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Biological Controls and Operations

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13.1

Biological Controls and Operations*

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13.1

Biological Controls and Operations

1.0 Introduction

This document addresses generic hazards and controls for biological operations at the Lawrence Livermore National Laboratory (LLNL). Biological operations include the use and safe handling of biohazardous materials, agents, or their components (e.g., microbial agents, bloodborne pathogens, recombinant DNA, Agricultural Pathogens, and human or primate cell cultures) and research proposals and activities concerning animal or human subjects.

Some biological operations require institutional review. The local environment, safety, and health (ES&H) representative can provide specific information for each operation.

This document applies to LLNL workers, hosted visitors, students, participating guests, contract laborers, supplemental personnel, and subcontractor workers where LLNL has management control.

2.0 Hazards

Biological operations often involve work with hazardous materials. Some individuals may have increased susceptibility to biohazards due to preexisting diseases, use of medications, compromised immunity, pregnancy, or breast-feeding. Such factors shall be addressed as part of the hazard assessment described in Document 2.2, "Managing ES&H for LLNL Work," in the *ES&H Manual*. For information regarding sanitation, refer to Document 13.3, "Sanitation," in the *ES&H Manual*.

Guidance documents, such as those listed below, are often used to determine the level of exposure to biological hazards.

- Centers for Disease Control (CDC)/National Institutes of Health (NIH), *Classification of Human Etiologic Agents on the Basis of Hazard*.

  http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm

- CDC/NIH, *Biosafety in Microbiological and Biomedical Laboratories*.

  http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

- National Cancer Institute, *Biosafety Manual for Research Involving Oncogenic Viruses*.

- NIH, *Guidelines for Research Involving Recombinant DNA Molecules.*

  http://www4.od.nih.gov/oba/rac/frnotices/1-5-01act.htm

Biological research and operations at LLNL are limited to Biosafety Levels (BSL) 1, 2, and 3, as defined by CDC/NIH. Activities that require BSL 4 precautions are prohibited.

2.1 Operations Involving Biological Materials

At LLNL, biological operations include the following:

- Healthcare.
- Emergency response.
- Laboratory research operations.
- Hazards Control Bioassay Laboratory and Hazards Control Whole Body Counting Laboratory.
- Plant Engineering maintenance and groundskeeping activities.
- Environmental surveillance.
- Facility restoration.
- Waste disposal operation
- Shipping and transportation.
- Animal handling.

2.1.1 Healthcare and Emergency Response

The biohazards involving human tissue and human body fluids and encountered in caring for ill or injured humans have been determined by the Occupational Safety and Health Administration (OSHA) to have the potential for contaminating workers with bloodborne pathogens. See 29 CFR 1910.1030, "Bloodborne Pathogens," for details. Bloodborne pathogens include, but are not limited to, the Hepatitis B virus (HBV), Hepatitis C virus (HCV), and the human immunodeficiency virus (HIV). For more information about bloodborne pathogens, also refer to Document 13.2, "Exposure Control Plan: Working Safely with Blood and Bloodborne Pathogens," in the *ES&H Manual* and Document 36.1, "Waste Management Requirements," in the *ES&H Manual*.

2.1.2 Laboratory Research Operations

Research operations may involve work with specific microbial (i.e., risk groups 1-3) agents, human tissue or body fluids, human or primate cell culture lines, or animals. Work with human or primate cell culture lines poses a hazard because the presence of latent viruses may exist incidentally or deliberately from experimental infections. Primary and permanent human or animal cell lines from nonlymphoid cell lines should be regarded as carrying low-hazard viruses unless known to be infected with a more hazardous agent(s). All primate cell lines derived from lymphoid cells, primate tumor tissue cell lines, primate cell lines exposed to or transformed by a primate oncogenic virus, primate cell lines contaminated with mycoplasma, and permanent human lymphocyte cell cultures are assumed to harbor moderate or higher hazard agents. Under no circumstances should anyone work with cells derived from himself/herself or a first-degree relative, since the host immune system may not provide adequate protection.

2.1.3 Plant Engineering Maintenance and Groundskeeping Activities

Sewage workers, plumbers, electricians, and other tradespersons, as well as janitors and gardeners, may come into contact with human body fluids or other potentially contaminated materials. Hazards to Plant Engineering maintenance and grounds workers include potential exposure to infectious agents that normally may be present in wild animals. Hazards may be contained in animal vectors, tissues, fluids, carcasses, or droppings.

2.1.4 Environmental Surveillance

LLNL and Site 300 drinking water may have physical, chemical, and biological contamination, such as low or high pH, residual chlorine level, bacteria total plate count, and fecal coliforms (e.g., E. coli). The sewer treatment process at Site 300 has the potential for introducing fecal coliform contamination into the sewer pond and groundwater.

Accidental releases into the environment may cause an ecological imbalance, which may have a negative impact on specific food industries and sewage-treatment facilities. An accidental release also may pose a threat to the health of the general public and to those individuals who may be immunologically compromised.

2.1.5 Facility Restoration

When replacing water-damaged materials (e.g., sheetrock, ceiling tiles, rugs, and siding), workers may be exposed to toxic fungal agents or their metabolites. Unoccupied or unused buildings may contain rodents or birds and their droppings, as well as poisonous snakes, insects, or spiders. The process of decontaminating facilities

that have been used for biological research or other work involving animals or human biological fluids may expose workers to biological agents or the effects of decontaminating agent.

2.1.6 Waste Disposal Operations

Workers who package and handle waste containing biological materials may be exposed to biohazards such as microbial agents and human or animal fluids or tissues.

2.1.7 Shipping and Transportation

Shipping or transport of biological materials, including microbial agents, human or animal fluids or tissues, animals, plants or plant pathogens, or biological waste, may result in worker exposure because of damaged shipping containers or inadequate packaging or handling.

2.1.8 Animal Handling

Research with animals poses hazards to the animals and to the handler. Hazards include allergic responses and illnesses from direct or indirect exposure to infectious agents and infectious test agents found in animal tissues, fluids, carcasses, or droppings. Exposure to such hazards may occur through dust inhalation, bites, and scratches or from handling cages or contact with waste materials.

2.2 Hazards Associated with Specific Agents and Materials

Biohazards are defined as biological agents, materials, or their components that are known or suspected to cause illness or injury to humans, animals, plants, or the environment. Of special interest are human fluids and tissues, which may contain bloodborne pathogens, including HBV, HCV, and HIV.

Biohazardous agents are organisms that have the capacity to produce deleterious effects because of their infectious nature. Examples of such agents include various viruses, chlamydia, bacteria, fungi, yeast, and algae. Such agents may be found in human blood, blood products, tissues, and certain body fluids; cultured cells; certain recombinant products; clinical specimens; and infected animals and animal tissues and droppings.

Biohazardous agents can be transmitted by many different pathways. Transmission can occur from human to human, animal to human, insects to human, or insect to animals through the air or by physical contact. Such agents may be found in water, food, soil, and biohazardous waste, in addition to human and animal sources. Humans may be exposed from inhalation, ingestion, skin absorption, and percutaneous routes.

Biohazardous materials are living components of biological agents that can or might cause illness or injury to humans, plants, and animals. Examples of biohazardous materials include toxins, metabolites, and other protein-based products that may initiate an allergic response.

3.0 Controls for Working with Biohazardous Materials

This section describes the three types of controls used at the Laboratory to prevent or mitigate the hazard(s) associated with biological operations and work involving biohazardous agents and materials:

- Administrative controls.
- Engineered controls.
- Personnel protective equipment.

By analyzing the hazards for each specific operation, LLNL personnel can develop and implement the appropriate controls to protect themselves, the community, and the environment from potential exposure.

Furthermore, the policies of funding and government agencies require managers of research and nonresearch activities to establish and review procedures for safe biological operations and the safe use and disposal of biohazardous materials. When biological activities or operations involve humans or live animals, additional regulations apply.

The classification of hazards and the terminology used to describe them vary from agency to agency, depending on the subject area. Table 1 contains a list of agencies and their operational requirements for work with microbial agents and toxins, animal care, and human subjects. Regulations included in the Work Smart Standards set that are specific to biohazardous operations are marked with an asterisk (*). Contact the ES&H Team for specific and additional information.

3.1 Administrative Controls

Administrative controls consist of the following:

- The hazard review process
- Procedures and operational controls

Table 1. Government agencies and their biological hazards authority.

Agency	Microbial Agents/Toxins	Animal Care	Human Subjects
I. Federal			
U.S. Department of Agriculture (USDA)	—	9 CFR 1–3, "Animal & Plant Health Inspection Service (APHIS) Animal Welfare Act of 1966" (PL89544) as pertaining to research	—
	7 CFR 331 "Possession, Use, and Transfer of Biological Agents and Toxins" (for Plants)		
	9 CFR 121 "Possession, Use, and Transfer of Biological Agents and Toxins" (for Animals)		
	Form VS16-3, "Importation and Transport of Controlled Organisms or Vectors"	Form VS17-129, "Importation of Live Animals"	
	Form VS16-7, "Additional Information for Cell Cultures and Their Products"		
U.S. Department of Commerce	15 CFR 742, 744, 744B, " Exportation Administration Regulations"	—	—
U.S. Department of Energy (DOE)	*DOE O 440.1A, "Worker Protection Management for DOE Federal Contractor Employees"	—	DOE O 443.1 "Protection of Human Subjects"
		—	DOE O 300.2C, "Work with Others"
U.S. Department of Health and Human Services (HHS), Public Health Service (PHS), National Institutes of Health (NIH)	*66 FR 1146, "Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)," January 5, 2001 "Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)," amended April 2002	—	45 CFR 46, "Protection of Human Subjects: The Common Rule"
	42 CFR 72, "Interstate Shipment of Etiological Agents"	PHS Policy on Humane Care and Use Lab Animals (reprinted in 1996)	—

Table 1. Government agencies and their biological hazards authority. (cont'd)

Agency	Microbial Agents/Toxins	Animal Care	Human Subjects
I. Federal (cont'd)			
U.S. Department of Health and Human Services (HHS), Public Health Service (PHS), National Institutes of Health (NIH) (cont'd)	42 CFR 71.54, "Foreign Quarantine: Etiologic Agents, Hosts, and Vectors"	–	–
	*42 CFR 73, "Possession, Use, and Transfer of Select Agents and Toxins"		
Centers for Disease Control (CDC)/NIH	*"Biosafety in Microbiological and Biomedical Laboratories," CDC/NIH HHS Pub CDC 93-8395	*"Biosafety in Microbiological and Biomedical Laboratories," CDC/NIH HHS Pub CDC 93-8395	–
	"Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets"	"Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets"	–
Food and Drug Administration (FDA)	21 CFR 600-680, "Biologics"	–	*21 CFR 50, "Protection of Human Subjects" *21 CFR 56, "Institutional Review Boards"
U.S. Department of Labor	*29 CFR 1910.1030, "Bloodborne Pathogens"	*29 CFR 1910.1030, "Bloodborne Pathogens" *29 CFR 1910.1450 , "Occupational Exposure to Hazardous Chemicals in the Laboratories"	–
National Institute of Occupational Safety and Health	–	"Criteria for a Recommended Standard Occupational Exposure to Waste Anesthetic Gases," Publication No. 77-140 (March 1977)	–
U.S. Department of Transportation	*49 CFR173.134(a)1, "Definitions: Infectious Substances"	*49 CFR 173.134(a)1, "Definitions: Infectious Substances"	*49 CFR 173.134(a)1, "Definitions: Infectious Substances"

Table 1. Government agencies and their biological hazards authority. (cont'd)

Agency	Microbial Agents/Toxins	Animal Care	Human Subjects
I. Federal (cont'd)			
U.S. Department of Health and Human Services (HHS), Public Health Service (PHS), National Institutes of Health (NIH) (cont'd)	42 CFR 71.54, "Foreign Quarantine: Etiologic Agents, Hosts, and Vectors"	—	—
	*42 CFR 73, "Possession, Use, and Transfer of Select Agents and Toxins"		
II. State			
California State Department of Health Services	*22 CCR 65600–65628, "Minimum Standards for Permitting Medical Waste Facilities"	—	—
	*Ca Health & Safety Code 117600–118360, Medical Waste Management Act		
	8 CCR 5193, "Bloodborne Pathogens"		

DNA = Deoxyribonucleic Acid

CFR = Code of Federal Regulations

* Work Smart Standard (WSS)

3.1.1 Hazard Review Process

When an Integration Work Sheet (IWS) is completed for a proposed activity, the activity automatically receives programmatic review and an ES&H review as part of the hazards analysis process. This review process, which may be subjective, assesses or evaluates the operation and other factors as part of the hazard reduction process. The hazard reduction process is based on the potential for adverse health effects on a healthy human adult and does not account for individuals who may have increased susceptibility to biohazards due to preexisting diseases, use of medication, compromised immunity, or pregnancy. The hazards for people with these conditions shall be evaluated individually. Under these situations, care shall be taken to ensure that an individual's privacy is not violated. Consult with the Health Services Department for assistance in matters of medical confidentiality.

The review process identifies potential health hazards and environmental concerns and covers factors such as virulence, pathogenicity, infectious dose, environmental stability, route of dissemination, communicability, work process operations, quantity, availability of vaccine or treatment, toxicity and allergenic host response. Such review also determines the controls necessary to minimize hazard and comply with orders and regulations. Some proposed biological activities require institutional review, as described in the next paragraph. Activities that require institutional review are identified by completing the IWS and in consultation with the ES&H Team.

Institutional Review. All research activities involving infectious materials, recombinant DNA technology, animals, or use of human subjects require an institutional review by the appropriate committee listed below. The ES&H Team industrial hygienist can provide more information on these committees.

Laboratory Biosafety Operations Committee (LBOC). The LBOC reviews all biological operations and research projects conducted at LLNL, or by LLNL employees, for consistency with ISMS principles. The LBOC operates in an advisory capacity only and provides direction to LLNL investigators and operators regarding the proper review processes to meet ISMS requirements.

Institutional Biosafety Committee (IBC). The Institutional Biosafety Committee (IBC) is charged with the oversight of all research and operational activities involving:

- Recombinant DNA.
- Artificial gene transfer.
- Biological agents (e.g., bacteria, viruses, protozoa, fungi).
- Toxins (natural and synthetic).

- Biological materials from human and animal sources (e.g., tissues, body fluids, cell cultures).

This oversight extends to the use of these biological materials by LLNL employees in onsite or offsite collaborative activities.

Specific details of the IBC's responsibilities are defined by the *NIH's Guidelines for Research Involving Recombinant DNA Molecules,*

http://www.niehs.nih.gov/odhsb/biosafe/nih/rdna-apr98.pdf

as well as by local ISMS Guidelines including Work Smart Standards and the *ES&H Manual*. The CDC's *Biosafety in Microbial and Biomedical Laboratories* (*BMBL*)

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

provides additional information on agent summaries, containment levels (biosafety levels 1-4). The *BMBL* also identifies appropriate work practices and equipment to ensure safe work practices in the laboratory

The IBC was established by the Director of the Laboratory in 1991, and operates through the Council on Biology and Biotechnology under the oversight of the Associate Director for the Biology and Biotechnology Research Program. The IBC functions as a peer review committee, focusing on the safe and legal use of biological materials. It is made up of LLNL and DOE employees and community members**.**

Institutional Review Board (IRB) for Human Research. Federal law and regulations require that any proposed research project involving human subjects shall first be reviewed and approved by the appropriate authorities. At LLNL, the IRB handles this review and the approval process. The Laboratory established the IRB as part of its assurance to the U.S. Department of Health and Human Services that LLNL will comply with all federal regulations for the protection of human research subjects.

For proposed work with human subjects, the IRB review shall:

- Identify the hazards associated with the research.
- Determine whether or not the hazards will be minimized to the extent possible.
- Identify the probable benefits to be derived from the research.
- Determine whether or not the hazards are reasonable in relation to the benefits to the subjects (if any) and the importance of the knowledge to be gained.
- Ensure that potential subjects will be provided an accurate and fair description of the hazards or discomforts and the anticipated benefits.

- Determine the intervals of periodic review.

In addition, the IRB should determine the provisions to protect the privacy of subjects and to maintain the confidentiality of data. Specific details on the IRB's review and the approval process can be found at

http://www.llnl.gov/HumanSubjects/

Institutional Animal Care and Use Committee (IACUC) for Animal Research. The IACUC shall review and approve any research involving warm-blooded vertebrate animals. The IACUC determines whether the proposed work meets acceptable standards for the humane care, treatment, and use of animals in research and protects the health and safety of workers. Assessment includes topics such as animal housing, handling, sanitation, nutrition, availability of water, veterinary care, protection from extreme weather and temperatures; waste disposal; and pest control. The IACUC is responsible for ensuring compliance with the Animal Welfare Act (AWA) and provides documentation to the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), which administers these standards and regulations.

Procedures and Operational Controls

Biosafety levels. For research involving microbial agents, four biosafety levels (BSL 1 through BSL 4) of containment have been established and are described in the NIH Guidelines and in the 4th edition (1999) of "Biosafety in Microbiological and Biomedical Laboratories." Agent Summary Statements for work with specific bacterial, fungal, parasitic, prion, rickettsial, and viral agents can also be obtained from the NIH reference listed above. The purpose of containment is to reduce or eliminate exposure of laboratory workers, visitors, and other persons, and the environment to potentially biohazardous agents. Containment has been classified into two major components: (1) a set of standard work practices that are generally used in microbiological laboratories and (2) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard.

The controls for biological activities include:

- General microbiological practices and techniques.
- Universal precautions.
- Good personal hygiene.
- Good housekeeping practices.
- Medical surveillance.
- Worker safety awareness and training.

- Warning signs.
- Procedures for
  - Decontamination.
  - Environmental surveillance.
  - Maintenance.
  - Shipping/transport of biohazardous materials.
  - Waste disposal.

The above controls are discussed in detail below.

General Microbiological Practices and Techniques. The most important element of containment in clinical and nonclinical settings is strict adherence to general microbiological practices and techniques (GMT). Listed below are a generic set of GMTs, which should be tailored to the specific operation and agents:

- Limit or restrict access to the laboratory when work is in progress.
- Wash hands after handling biological materials and animals but before exiting the area.
- Do not eat, drink, smoke, apply cosmetics, handle contact lenses, or store food in the laboratory area.
- Do not mouth pipette; use mechanical pipetting device(s).
- Minimize the creation of droplets, spatter, splashes, or aerosols.
- Use sharp equipment (e.g., hypodermic needles, syringes, and scalpels) only when necessary. Do not cut or bend hypodermic needles. Place all sharps in the designated sharps container for further treatment and disposal. All syringes used in the care of the ill or injured or in healthcare environments shall be safety syringes. Syringes shall never be recapped.
- Decontaminate work surfaces before and after each workday and after any spill of viable materials. See Appendix C for details.
- Decontaminate or autoclave all cultures, stocks, and other biological waste before disposal.
- Properly label all containers containing biological material.
- Put into effect an insect and rodent control program.

Universal Precautions. Below is a standard set of OSHA-promulgated precautions that specifically apply to the handling of human blood; human blood products; some human body fluids; some unfixed tissues and organs; HIV-containing cell or tissue cultures, HIV organ cultures, and HIV- or HBV -containing culture medium or other solutions; and blood, organs or other tissues from experimental animals infected with HIV or HBV.

- Routinely use gloves, masks, protective eyewear, face shields, protective clothing, and so forth, to prevent skin and mucous membrane contact and contamination from bloodborne pathogens.
- Wash hands or other skin surfaces thoroughly and immediately if contamination occurs and after removal of gloves or protective clothing.
- Handle, use, and dispose of sharp objects carefully to avoid accidental injuries.
- Use mouthpieces, resuscitation bags, or other ventilation devices when resuscitation is needed.
- If you have exudative lesions or weeping dermatitis, refrain from all direct patient care and from handling patient-care equipment until the condition is resolved.

Personal Hygiene. Personal hygiene is important in reducing or minimizing exposure. Loose hair and clothing shall be confined in work areas where the potential for exposure to hazardous materials exists. Workers are encouraged to wash hands frequently with water and a mild soap or with an antiseptic cleanser whenever skin comes in contact with hazardous or infectious materials. Hands should always be washed before leaving the work area.

Good Housekeeping Practices. Floors shall be cleaned and swept regularly. All aisles, hallways, and stairs shall be kept clear. Access to emergency equipment and exits shall be clear and unobstructed.

Medical Surveillance. The responsibility for medical surveillance belongs to the work supervisor (if matrixed, the work supervisor should notify the payroll supervisor who has the responsibility for ensuring medical surveillance). The work supervisor shall consult with the ES&H Team to identify those workers who may be recommended for medical surveillance, possibly to include immunization. Serum banking may also be recommended.

Safety Awareness and Training. Workers whose primary job is to handle biological materials shall be trained at the time of initial assignment. Work supervisors shall provide additional training for specific job duties. For more information regarding training requirements for work with blood or bloodborne pathogens, see Document 13.2. For more information regarding the training requirements for work

with biohzardous agents, see Document 13.6, "Safe Handling and Use of Biological Research Materials," in the *ES&H Manual*. All formal training completed by the worker, such as HS 4400, "Working Safely with Blood and Bloodborne Pathogens," shall be documented and maintained in the Laboratory Training and Information Network (LTRAIN). All other training required by the respective Directorates should be documented and maintained within the program directorate.

The Canadian government's Health Canada Internet site

http://www.hc-sc.gc.ca/pphb-dgspsp/msds-ftss/index.html

lists about 150 material safety data sheets (MSDSs) for infectious microorganisms. These MSDSs include the following information:

- Infectious dose.
- Viability.
- Mode of transmission and dissemination.
- Decontamination methods.
- Medical surveillance, first aid/treatment, immunization, and prophylaxes.
- Recommended precautions.
- Handling during spills, disposal, and storage.

Check with your ES&H Team industrial hygienist before planning work with any microorganisms.

Additional supplementary background information is also available at the following websites:

- Health Canada:
  http://www.hc-sc.gc.ca/hpb/lcdc/biosafty/index.html
- Belgian Biosafety Server:
  http://biosafety.ihe.be/
- Centers for Disease Control and Prevention:
  http://www.cdc.gov/od/ohs/

Warning Sign. The biohazard symbol, normally red on a white background (see Fig. 1), shall be used as a warning sign on all facility access doors where infectious agents are present. The sign should indicate the name of the biohazard(s) present, the person(s) responsible for the work, and any restrictions on access to the room.

Figure 1. Biohazard sign.

Decontamination. Biological agents and toxins can be contained by the process of decontamination. The three types of decontamination are sanitation, disinfection, and sterilization, defined as follows:

Sanitation	The physical or chemical removal or reduction in the number of microorganisms by use of general cleaning agents.
Disinfection	The inactivation of targeted organisms with physical or chemical agents.
Sterilization	The total destruction of all microorganisms, including highly resistant bacterial endospores.

Table 2 summarizes liquid, gaseous, and physical decontaminating agents for sanitation, disinfection, and sterilization.

Environmental Surveillance. LLNL and Site 300 drinking water is monitored weekly to evaluate potential physical, chemical, and biological contamination, such as low or high pH, residual chlorine level, bacteria total plate count, and fecal coliforms (e.g., E. coli). To determine the effectiveness of the sewer-treatment process at Site 300, the sewer pond is monitored periodically and the groundwater semiannually for fecal coliform.

Emergency Response to Spills and Releases. The possibility of a spill exists in any operation. Because of the serious ramifications for worker exposure and accidental releases into the environment, control measures to prevent such events from occurring are now mandated by law. Although the severity of a potential accident dictates the response required, the general approach in responding to emergency spills is listed below in order of priority. For more information, see Appendix C.

- Protect personnel.
- Decontaminate affected area.
- Repair or replace contaminated equipment.

Table 2. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins.

Decontaminating agent	No effect on	Sanitize[a]	Disinfect[a]	Sterilize[a]
Liquids				
Alcohol, ethyl (70–95% v/v)	Bacterial spores[1]	—	M tuberculosis (TB)[1] at 95% for 0.25 min	—
			TB[2] at 70% for 0.5 min	—
	Nonlipid viruses[3]	—	Lipoviruses for 1.0 min	—
	—	—	At 50% for 2.0 min	Medical instruments[1] at 70% for 15 min
Alcohol, isopropyl (60–90% v/v)	Bacterial spores[2]	At 70% for <2 min	At 70% for 2 min	At 70% for >5 min
Alcohol, Isopropyl +5% propylene oxide	—	—	—	Bacterial spores[1] for 1.0 min
Glutaraldehyde[1]	—	At <2% for 15 min	Bacillus anthrax at 2% for 15 min	At >2% for 15 min
	—	—	Cl. and Bacillus spores[1] at 2–3% for 180 min	—
Hydrogen peroxide	—	At <3% for 10 min	At 3–6% for 10 min	At 6–25% for 10
	—	—	10^8 bacterial spores[1] at 10% for 60 min	—
Phenol (1–5%)[3]	Bacterial spores,[3] Nonlipid viruses	At 0.5% for <30 min	Broad spec. at 0.5–3% for 30 min C. burnetti, F. turalensis, P. mallei, Bacillus anthracis, and Rickettsia at 5% for 30 min	At >3% for 30 min

Table 2. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins. (cont'd)

Decontaminating agent	No effect on	Sanitize[a]	Disinfect[a]	Sterilize[a]
Liquids (cont'd)				
Soap and water	–	C. burnetti[b,4] for 10 min. The toxins will be diluted if washed for more than 10 min.	Synergistic with certain phenol derivatives	–
Sodium hypochlorite, (household bleach) undiluted	–	–	–	Cl. botulinum toxin, SEB, and Ricin[4] at 250 ppm (0.5%) for 15 min
	–	At <50 ppm (<0.1%) for 30 min	Most bacterial toxins at 50 ppm (0.1%) for 30 min TB at 50 ppm (0.1%) for for 30 sec at pH of 8.4 at 50–60°C General bacteria and viruses at 500 ppm (1.0%) for 30 min Bacillus anthracis[1] at 2500 ppm (5%) for 30 min	Lipoviruses at 500 ppm (1.0%) for 30 min
	–	–	–	SEB at 250 ppm (0.5%) for 72 min T2 mycotoxin at 2500 ppm (5%) for 72 min
Gaseous Agents				
Paraformaldehyde[1]	–	At <1% for 60 min	At 2% for 60 min	SEB[5] at >2% for 60 min
	–	–	Cl. spores and Bacillus spores at 8% for 180 min	–

Table 2. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins. (cont'd)

Decontaminating agent	No effect on	Sanitize[a]	Disinfect[a]	Sterilize[a]
Gaseous Agents (cont'd)				
Ethylene oxide (12% EtO: 88% propellant)	—	—	—	At 130°F for 1200 mg/L for 120 min At 130°F at 650 mg/L for 240 min At 130°F at 470 mg/L for 320 min At 100°F at 470 mg/L for 480 min
Vapor-Phase Hydrogen Peroxide at 2 mg/L (20%)	Porous materials	—	—	B. globigii for 0.17 min Cl. sporogenes for 0.20 min B. Stearothermophilus for 0.30 min
Physical Agents				
Steam heat				
At 80°C	—	—	VEE, SEB, and Ricin for 30 min	—
At 121°C	Saxitoxin, and Tetrodotoxin	—	—	All bacteria, lipoviruses, protein-based toxins, and viruses for 15 min
At >500°C	—	—	T2 mycotoxin for 30 min	—
Gamma radiation (Cobalt 60)	—	—	—	S. faecium: <50 organisms/item at 3.2 Mrad; 50–500 organisms/item at 4.5 Mrad; and 500–5000 organisms/item at 5.0 Mrad

Table 2. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins. (cont'd)

Decontaminating agent	No effect on	Sanitize[a]	Disinfect[a]	Sterilize[a]
Physical Agents (cont'd)				
Ultraviolet (UV) light (180–280 nm UV-C)	Cl. botulinum toxin, bacterial spores, Ebola virus, slow-growing viruses, lipoviruses, Micrococcus radiodurans, and non-protein-based toxins	At 0.5 mW/cm^2 for 1440 min (24 h) (Ref. 1)	Biological safety cabinets, for example, at 35–40 mW/cm^2 for 30 min	T2 mycotoxin and protein-based toxins at >40 mW/cm^2 for 30 min
X ray	Bacterial spores, Micrococcus radiodurans, and Micrococcus radiophilus	—	Salmonella typhimurium at 0.65 Mrad (6500 Gy)	Medical devices[1] at 2.5 Mrad (25,000 Gy)

a Contact times may vary and are dependent on the concentration of agent used.

b Synergistic with sodium palmitate, sodium stereate, sodium ricinate, coconut soap, and castile soap.

Soap and water will often dilute contaminants from equipment and skin.

1 Seymour S. Block, *Disinfection, Sterilization, and Preservation*, 3rd edition, Lea & Febiger (1983), p. 1053.

2 59 FR 34496, *Guidelines for Research Involving Recombinant DNA Molecules*, July 5, 1994. Also see the following Internet address: http://www.nih.gov/od/oba/rdna.htm/

3 American Industrial Hygiene Association, *Biosafety Reference Manual*, 2nd edition, Publication No. 204-RC-95 (1995), p. 175.

4 Franz, David, *U.S. Army Defense Against Toxin Weapons*, 2nd edition, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD (August 1996).

5 *U.S. Army Field Manual* 3-9, "Potential Military Chemical/Biological Agents and Compounds" (December 12, 1990).

Maintenance. Plant Engineering maintenance and grounds workers shall take precautions to minimize potential exposure to biohazardous agents that may be transmitted from animals, insects, and reptiles. Bacteriocides are commonly used in cooling towers, evaporative coolers, and swamp coolers to control the microbial population.

Shipping/Transport/Receiving. To prevent and minimize exposure to biohazards, biological materials, including waste, are transported in properly labeled primary and secondary leak-proof containers. Materials transported in public areas within the contiguous boundaries of the Laboratory shall be placed in a properly labeled secondary container that is closable and lockable (i.e., 6-pack Igloo cooler). Animals are transported in wired, filtered cages.

Transportation of biological materials to offsite locations shall be in accordance with U.S. Department of Transportation, U.S. Department of Commerce, Centers for Disease Control and Prevention, U.S. Department of Agriculture, and public health regulations, depending on the materials. See Table 1 for the appropriate regulations. Importers of select agents shall comply with the CDC's regulation (42 CFR 71.54, "Etiological Agents, Hosts, and Vectors"). Exporters shall follow the Department of Commerce exportation regulation (15 CFR 742, 744, and 774). Contact the LLNL Transportation Department for more information.

The Material Distribution Division (MDD) shall receive all incoming shipments of infectious substance(s) and etiologic agent(s) according to OP 301.1, "Basic Receiving and Distribution," which can be found at the following Internet address:

http://www-r.llnl.gov/pm/mdd/pdf/p301.1.pdf

Packages containing such substance(s) bear one of the following labels:

INFECTIOUS SUBSTANCE
IN CASE OF DAMAGE OR LEAKAGE
IMMEDIATELY NOTIFY PUBLIC HEALTH AUTHORITY
IN U.S.A. NOTIFY DIRECTOR – CDC
ATLANTA, GA. (404) 633-5313

ETIOLOGIC AGENTS
BIOMEDICAL MATERIAL
IN CASE OF DAMAGE OR LEAKAGE
NOTIFY DIRECTOR CDC
ATLANTA, GA (404) 633-5313

MDD shall secure the substance in the receiving cage and notify the requester or qualified designee to arrange for it to be picked up within one hour.

Waste Disposal. Biohazardous waste generated from research laboratories and clinical settings should be placed in a primary autoclave bag within a labeled, durable, leak proof secondary container with a closable lid. Biohazardous or biological waste from animals, including animal waste, droppings, and carcasses, shall be carefully segregated and disposed of based on the hazard present. For example, radioactive biohazardous or biological waste shall be disposed of as radioactively contaminated waste. Chemically contaminated biohazardous or biological waste shall be disposed of as biohazardous or biological waste. For additional information on medical waste, see Document 36.1, "Waste Management Requirements," in the *ES&H Manual*.

Containment. Work in research or production facilities involving quantities of materials greater than 10 L of culture will require special physical containment as specified by the IBC. The maximum quantity handled at any time in BSL-3 laboratories will be less than 1 liter of cultured microorganisms. The degree of containment is based on the level of health hazard or the level of impact the agent being studied poses to personal work practices and the environment.

3.2 Engineered Controls

Engineered controls are varied to include biological safety cabinets, high efficiency particulate air (HEPA) filters, sinks with foot-actuated taps, easy-to-clean surfaces, HVAC systems that protect workers and the environment from contaminated airflow, appliances that minimize aerosol production, and security controls. Some of these engineered controls are discussed below. For more information on designs of engineered controls, refer to *Biosafety in Microbiological and Biomedical Laboratories* (listed in Section 5.0, "Work Smart Standards").

3.2.1 Facility Design

Examples of facility design controls are HEPA filters, interlocks, and negative airflow units. Recommended facility design controls depend on the risk of transmission of specific biohazardous agents.

3.2.2 Safety Equipment

Safety equipment includes mechanical aids (e.g., tongs and tweezers), dead air boxes, sharps containers, laboratory-type fume hoods, biological safety cabinets (also referred to as "biosafety cabinets"), shielding, safety centrifuge cups, and special shipping containers for transporting biological materials and animals. Biological safety cabinets are discussed in detail below.

Biological Safety Cabinets. Ventilation control of infectious agents or other biologically derived molecules is usually achieved by performing the operation using a biological safety cabinet. There are currently three primary classes of biological safety

cabinets (Classes I, II, and III). Each class is distinguished by its design and its containment and cleanliness capability. The selection of an appropriate biological safety cabinet for a given operation shall be approved by the area ES&H Team industrial hygienist based on the specifics of the operation and an evaluation of the biosafety level classification (i.e., BSL 1, 2, 3). (For a definition of biosafety levels, see Appendix A.)

Class I cabinets are similar to a conventional laboratory hood with an open-face and negative-pressure design.

Class II cabinets, commonly referred to as laminar-flow biological safety cabinets, are effective in protecting operators from research materials as well as protecting research materials from external contamination. The Class II cabinet design utilizes a HEPA filter in an overhead diffuser to reduce contamination in the cabinet. Air flows down toward the work surface and through two separate grills (front and rear). An air barrier for the protection of operators is created by the airflow into the cabinet from the room and the down flow air through the front grill. There are four basic types of Class II cabinets: Types A, B1, B2, B3, and C. Each cabinet type has differences that include:

- Proportion of air recirculated into the work area.
- Airflow velocities into the work opening and down toward the work surface.
- Manner of exhaust air discharge.
- Air plenum pressure relative to the room.

Class III cabinets are hermetically sealed enclosures for the handling of extremely hazardous materials at Biosafety Level 4. No Biosafety Level 4 work is currently authorized at LLNL.

Further descriptions of Class II and III cabinets, including schematic diagrams, can be found in the following documents:

- CDC/NIH, *Biosafety in Microbiological and Biomedical Laboratory*.

  http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

- CDC/NIH, *Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets*.

  http://www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm

- American Industrial Hygiene Association, *Biosafety Reference Manual*.

Design specifications shall be approved by the ES&H Team industrial hygienist. Table 3 lists the recommended minimum performance guidelines for biological safety cabinets.

Table 3. Performance guidelines for biological safety cabinets.

Biological safety cabinet	Description	Minimum face velocity (fpm)*	Negative pressure (inches w.g.) *
Class I	Front panel not in place	80	N/A**
	Front panel without gloves	150	N/A
	Front panel with gloves	N/A	0.5
Class II, Type A	Fixed opening height, usually 10 inches	80	N/A
Class II, Type B and 100% exhaust	Sliding sash adjustable from 8–30 inches. Experiment should be performed with 8-inch opening for proper face velocity.	100 (at 8-inch opening)	N/A
Class III	No direct opening. Access through double door sterilizer and decontaminant dunk bath.	N/A	0.5

* The manufacturer provides specifications for certain biological safety cabinets that obtained National Sanitation Foundation certification. The information in this table is not always applicable.

** N/A = not applicable.

Design considerations for the use of biosafety cabinets in a research setting are similar to those for laboratory hoods as described in Document 12.4, "Work Enclosures and Local Exhaust Systems for Toxic and Radioactive Materials," in the *ES&H Manual.* Design, construction, and performance for Class II biological safety cabinets have been developed by the National Sanitation Foundation International (NSF). The performance of all biosafety cabinets shall be tested and evaluated in accordance with NSF Standard 49 for Class II Biosafety Cabinets by a certified biological safety cabinet certifier:

- After a cabinet is purchased and installed but before use.
- After a cabinet is moved, relocated, or serviced.
- Annually if the cabinet has not otherwise been inspected that year.

3.3 Personal Protective Equipment

Personal protective equipment (PPE) includes gloves, coats, gowns, shoe covers, safety shoes, boots, respirators, face shields, and safety glasses or goggles. PPE is to be used only as supplemental protection if there is still a residual risk of exposure after engineered and administrative controls are implemented. Identifying, reviewing, and assessing the workplace hazard(s), activities, and operations are key to selecting effective and appropriate PPE. Choice of PPE, and the required training for its use is determined on a case-by-case basis by the ES&H Team Industrial Hygienist. Respiratory protection is required for certain animal work at the BSL-3 level.

Closed-toed shoes shall be worn to protect the feet from common laboratory hazards (e.g., acids, bases, solvents, and broken glass). Sandal-type shoes are prohibited in areas where such hazards exist. See Document 11.1, "Personal Protective Equipment," in the *ES&H Manual* for more PPE information.

4.0 Responsibilities

The job responsibilities for individuals who supervise or work with biological materials are described in the subsections that follow. General responsibilities for all staff levels are described in Document 2.1, "Laboratory ES&H Policies, General Worker Responsibilities, and Integrated Safety Management," in the *ES&H Manual*.

4.1 Workers

- Conduct each task in accordance with the applicable safety plans and established institutional procedures.
- Attend required training sessions before beginning specific tasks involving blood or other potentially infectious materials.
- Participate in medical and immunization program, if desired.
- Use PPE and other protective devices when required.
- Immediately report any occupational exposure to the Health Services Department and the Hazards Control Department.

4.2 Responsible Individuals

- Screen work using the IWS and coordinate or prepare if necessary the hazard assessment control (HAC) form (or equivalent) .
- Obtain approval from appropriate institutional committee (i.e., Biosafety, Human Subjects or Animal Care and Use) as necessary. For more information, contact the chair of the respective committee.
- Identify and develop safety plans (e.g., Safety Plans [SPs], Facility Safety Plans, and Bloodborne Pathogen Exposure Control Plans) when work activities involve the use of biological materials (e.g., blood and other potentially infectious materials).
- Plan activities so as to assure the safe use of biological materials.
- Perform ES&H evaluations in coordination with the ES&H Team.

- Prepare emergency, waste disposal, and decontamination plans.
- Provide PPE to individuals who work with biological materials.
- Ensure that workers know and follow the requirements in Document 13.2, as well as those in this document when working with biological materials**.**
- Ensure proper implementation of the engineered and administrative controls specified in this document.
- Ensure that identified workers complete a medical surveillance and immunization program.
- Ensure that PPE, other devices, and clothing are available and functional.
- Obtain concurrence for all hazardous operations involving the handling of biological materials from the industrial hygienist and environmental analyst assigned to your ES&H Team, as well as authorization from the program manager or division leader.
- Ensure that provisions of any applicable CDC/USDA permits are understood and implemented.

4.3 Health Services Department

- In collaboration with the Hazards Control Department, provide a medical surveillance and immunization program as necessary for workers who handle biological materials.
- Provide medical treatment of significant exposures and worksite assessment to reduce the risk of subsequent exposures.
- Maintain the medical records of workers who work with blood or other potentially infectious materials, including a record of immunization, surveillance, and post-exposure assessment and treatment.

4.4 Hazards Control Department

- Assist in the identification of hazards associated with biological materials or other potentially infectious materials.
- Assist workers in working safely with biological materials or other potentially infectious materials.
- Concur on hazards assessments for operations involving biological agents or potentially infectious materials.

- Determine the need for and frequency of workplace monitoring and inform supervisors, workers, and the Health Services Department of the results.
- Identify required PPE for all workers in biohazardous areas.
- Provide specific training to programs and divisions, as required.

4.5 Environmental Protection Department

4.5.1 Environmental Analyst

- Concur on the hazard assessments for operations involving the use of blood or other potentially infectious materials.
- Provide guidance to biohazard handlers on how to implement environmental controls and procedures and on the proper management of hazardous waste contaminated with biohazards to ensure compliance with all applicable federal, state, and local environmental requirements.
- Provide specific training to programs and divisions, as required.

4.5.2 Hazardous Waste Management Technician

- Provide specific guidance to biohazard handlers on how to properly segregate, package, and label solid and liquid wastes that are contaminated with blood or other potentially infectious materials.
- Coordinate the disposal of biological waste generated in the area.

5.0 Work Standards

22 CCR 65600–65628, "Minimum Standards for Permitting Medical Waste Facilities."

http://www.calregs.com/

7 CFR 331 and 9 CFR 121, Animal and Plant Health Inspection Service; Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins; Interim Final Rule

http://www.cdc.gov/od/sap/docs/btarule.pdf

9 CFR Chapter 1 (the Animal Welfare Act of 1966), Subchapter A, "Animal Welfare," Parts 1, 2, and 3 (P.L. 89544).

http://www.aphis.usda.gov/reac

9 CFR Chapter I, Part 104, Permits for Biological Products

http://www.access.gpo.gov/nara/cfr/waisidx_01/9cfr104_01.html

29 CFR 1910.1030, "Bloodborne Pathogens," including Needlestick Safety and Prevention Act, January 18, 2001.

http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051

29 CFR 1910.1450, "Occupational Exposure to Hazardous Chemicals in Laboratories."

http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10106&p_table=STANDARDS

42 CFR 73, "Possession, Use, and Transfer of Select Agents (for Humans)."

http://www.cdc.gov/od/sap/docs/42cfr73.pdf

45 CFR 46, "Protection of Human Subjects, Subpart A, B, C, and D."

http://www.fas.harvard.edu/~research/45CFR46.html

49 CFR Parts 100–185, 199, "Hazardous Materials Regulations: Toxins/Microbes," Subtitle B, Chapter I, "Research And Special Programs Administration, DOT."

http://www.myregs.com/dotrspa/21 CFR 50, "Protection of Human Subjects."http://www.fda.gov/oc/ohrt/irbs/appendixb.html

21 CFR 56, "Institutional Review Boards."

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=5666 FR 1146,

NIH Guidelines for Research Involving Recombinant DNA Molecules, January 5, 2001

http://www4.od.nih.gov/oba/rac/frnotices/1-5-01act.htm

http://www.nih.gov/od/orda/toc.htm

Biosafety in Microbiological and Biomedical Laboratories, 4th edition, U.S. Depart. of HHS, Public Health Service, HHS Publication No. (CDC) 93-8395, May 1999.

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

6.0 Resources for More Information

6.1 Contacts

Contact the following individuals or committees for more information about the topics discussed in this document:

- Directorate safety officer, assurance manager, Institutional Responsible Official (RO), or the institutional biosafety officer.
- Your area ES&H Team industrial hygienist or physician.
- Institutional Biosafety Committee—for questions about select agents and recombinant DNA experiments.
- Institutional Review Board—for questions about research involving human subjects.
- Institutional Animal Care and Use Committee for Animal Research—for questions about animal research.
- Environmental Protection Department EOG representative.
- Select Agent Responsible Official (RO) for questions about the LLNL Select Agent Program.

6.2 Other Sources

7 CFR 331 "Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins (for Plants)," December 13, 2002.

15 CFR 742, 744, 744B, "Exportation Administration Regulation."

21 CFR 600-680, "Biological Products."

http://www.tomescps.com/toc/fed/cfr/21/21_toc.html

42 CFR 71.54, " Etiological Agents, Hosts and Vectors."

42 CFR 72, "Interstate Shipment of Etiological Agents."

DOE O 300.2C, "Working with Others."

DOE Order 440.1A, "Worker Protection Management for DOE Federal and Contractor Employees," Attachment 2, "Contractor Requirement Document," Sections 1-11, 13-18 (delete item 18.a), 19 (delete item 19.d.3) and 22.

DOE O 443.1 "Protection of Human Subjects."

American Industrial Hygiene Association, *Biosafety Reference Manual*, 2nd edition, Publication #204–RC–95, (1995), pp. 175.

Seymour S. Block, *Disinfection, Sterilization, and Preservation*, 3rd edition, (Lea & Febiger Publishers, 1983), pp. 1053.

David Franz, *U.S. Army Defense Against Toxin Weapons* (U.S. Army Medical Research Institute of Infectious Diseases, Veterinary Corps, Fort Detrick, Fredrick, Maryland), MCMR-UIZ-B.

Guidelines (Draft) for Preventing the Transmission of Tuberculosis in Healthcare Facilities, FR58:195 (October 12, 1993).

National Cancer Institute, *Safety Standards for Research Involving Oncogenic Viruses*, DHEW, Publication No. NIH 75-790 (1974).

NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), April 2002.

NIOSH, *Criteria for a Recommended Standard Occupational Exposure to Waste Anesthetic Gases and Vapors*, DHEW Publication No. 77-140.

http://www.cdc.gov/niosh/77-140.html

NIOSH, *Histoplasmosis: Protecting Workers at Risk*, Publication No. 97-146.

http://www.cdc.gov/niosh/hi97146.html

U. S. Army, *U.S. Army Field Manual 3–9: Potential Military Chemical/Biological Agents and Compounds*, Navy Publication No. P-467, Air Force Manual 355-7 (December 12, 1990).

U.S. Army, *Medical Management of Biological Casualties Handbook*, Second Ed., (U.S. Army Medical Research, Institute of Infectious Diseases, Fort Detrick, Fredrick Maryland, August 1996).

U.S. Department of Public Health, National Institutes of Health, *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, reprinted 1996.

Appendix A

Terms and Definitions

APHIS	Animal and Plant Health Inspection Service. A component of the U.S. Department of Agriculture (USDA) that administers the Animal Welfare Act and oversees compliance with the USDA Select Agents Program and the importation of agents and materials that may pose a threat to U.S. agriculture. Within APHIS, Animal Care is the agency that is responsible for ensuring compliance with the animal welfare regulations.
Biohazardous agent	A living organism that has the capacity to produce deleterious effects because of its infectious nature. Biohazardous agents include, but are not limited to, various viruses, chlamydia, bacteria, fungi, yeast, and algae, as well as plants and animals and their products that contain any of these agents.
Biohazardous material	A live component of a biological agent that can or might cause illness or injury to humans, plants, and animals.
Biohazard	Any biological material or its components that present a real hazard of illness or injury to humans, plants, and animals.
Biosafety (containment) level	The level of containment required to perform biohazardous operations safely. Work practices and techniques, safety equipment, and laboratory facilities appropriate for the operations are based on the potential hazards imposed by the agents used and for the laboratory function and activities.
Biological safety cabinet (BSC)	An engineered control designed to enable laboratory workers to handle infectious etiologic agents safely and to provide primary containment of any resultant aerosol.
Blood	Human blood, human blood components, and products made from human blood.

Bloodborne pathogen	Any pathogenic microorganism that is present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).
CDC	Centers for Disease Control and Prevention. An agency of the Department of Health and Human Services. CDC oversees compliance with the CDC Select Agents Program and the importation of agents and materials that may pose a threat to U.S. public health.
Contaminated	The presence, or reasonably anticipated presence, of blood or other potentially infectious materials on an item or surface.
Decontamination	The use of physical or chemical means to remove, inactivate, or destroy pathogens on a surface or item to the point where the pathogens are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.
Engineered control	A control (e.g., biosafety cabinet, sharps disposal container, and self-sheathing needle) that isolates or removes a pathogen hazard from the workplace. For purposes of this document, this term is the same as OSHA's term "engineering control."
Etiologic agent	A viable microorganism or its toxin that causes, or may cause, human disease.
FDA	Food and Drug Administration. An agency of the Public Health Service.
Hand washing facility	A facility providing an adequate supply of running potable water, soap, and single-use towels or hot air drying machines.
HBV	Hepatitis B virus.
HIV	Human immunodeficiency virus.
IBC	Institutional Biosafety Committee. Oversees biosafety research.

IRB	Institutional Review Board. Oversees research involving human subjects.
LBOC	LLNL Biosafety Operations Committee. An advisory committee that provides guidance to investigators regarding assessments and approvals required for a protocol to be conducted at or under the auspices of LLNL.
NIH	National Institutes of Health. An agency of the Public Health Service.
Occupational exposure	Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of a worker's duties.
Oncogenic virus	A virus that causes cancer.
Other potentially infectious material (OPIM)	(1) Any of the following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids. (2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead). (3) An HIV-containing cell or tissue culture, organ culture, or HIV- or HBV-containing culture medium or other solution; blood, organs, or other tissues from experimental animals infected with HIV or HBV.
Pathogen	Any agent (usually living) capable of producing disease.
Personal protective equipment (PPE)	Specialized clothing or equipment worn by a worker for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts or blouses) not intended to function as protection against a hazard are not considered to be PPE.
Production facility	A facility engaged in industrial-scale, large-volume, or high-concentration production of microorganisms.

Research laboratory	A laboratory producing or using research-laboratory-scale amounts of biological materials or chemicals.
Select agent	A microorganism or toxin listed in 42 CFR 73, (Possession, Use and Transfer of Select Agents and Toxins; Interim Final Rule) Or in 7 CFR 331 or 9 CFR 121 (Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins; Interim Final Rule) and not subject to the current rules of exemption.
Sharp	Any object that can penetrate the skin, including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed metal edges (e.g., dental wires) that can result in an exposure incident.
Sterilize	The use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.
Universal precautions	An approach to infection control. According to the concept of universal precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.
Work practice	A control that reduces the likelihood of exposure by altering the manner in which a task is performed.
Zoonotic agent	A biological agent that causes animal diseases.

Appendix B

APHIS Plant Pathogens, HHS Select Infectious Agents, and USDA High Consequence Livestock Pathogens or Toxins

Viruses

1. African horse sickness virus β
2. African swine fever virus β
3. Akabane virus β
4. Avian influenza virus (highly5. pathogenic) β
5. Blue tongue virus (exotic) β
6. Camel pox virus β
7. Cercopithecine herpes virus (Herpes B virus) Ψ
8. Classical swine fever virus β
9. Crimean-Congo haemorrhagic fever virus Ψ
10. Eastern equine encephalitis virus χ
11. Ebola viruses Ψ
12. Foot and mouth disease virus β
13. Goat pox virus β
14. Japanese encephalitis virus β
15. Lassa fever virus Ψ
16. Lumpy skin disease virus β
17. Malignant catarrhal fever β
18. Marburg virus Ψ
19. Menangle virus β
20. Monkeypox virus Ψ
21. Newcastle disease virus (exotic) β
22. Nipah and Hendra complex viruses χ
23. Peste des petits ruminants β
24. Plum pox potyvirus α
25. Rift Valley fever virus χ
26. Rinderpest virus β
27. Sheep pox β
28. South American haemorrhagic fever viruses [(Junin, Machupo, Sabia, Flexal, Guanarito)] Ψ
29. Swine vesicular disease virus β
30. Tick-borne encephalitis complex (flavi) viruses [Central European Tick-borne encephalitis, Far Eastern Tick-borne encephalitis (Russian Spring and Summer encephalitis, Kyasanur Forest disease, Omsk Hemorrhagic Fever)] Ψ
31. Variola major virus (Smallpox virus) and Variola minor (Alastrim) Ψ
32. Venezuelan equine encephalitis virus χ
33. Vesicular stomatitis virus (exotic) β

Prion

1. Bovine spongiform encephalopathy agent β

Toxins

1. Abrin Ψ
2. Botulinum neurotoxins χ
3. *Clostridium perfringens* epsilon toxin χ
4. Conotoxins Ψ
5. Diacetoxyscirpenol Ψ
6. Ricin Ψ
7. Saxitoxin Ψ
8. Shigatoxin and Shiga-like ribosome inactivating proteins χ
9. Staphylococcal enterotoxins χ
10. Tetrodotoxin Ψ
11. T– 2 toxin χ

Bacteria

1. *Bacillus anthracis* χ
2. Botulinum neurotoxin producing strains of *Clostridium* χ
3. *Brucella abortus* χ
4. *Brucella melitensis* χ
5. *Brucella suis* χ
6. *Burkholderia mallei* χ
7. *Burkholderia pseudomallei* χ
8. *Coxiella burnetii* χ
9. *Cowdria Ruminantium* (Heartwater) β

10. *Francisella tularensis* [χ]
11. *Liberobacter africanus, Liberobacter asiaticus* [α]
12. *Mycoplasma capricolu/M.* F38*/M. mycoides capri* (contagious caprine pleuropneumonia agent) [β]
13. *Mycoplasma mycoides mycoides* (contagious bovine pleuropneumonia agent) [β]
14. *Ralstonia solanacearum* Race 3 [α]
15. *Rickettsia prowazekii* [Ψ]
16. *Rickettsia rickettsii* [ψ]
17. *Xanthomonas oryzae* pv. oryzicola [α]
18. *Xylella fastidiosa* (citrus variegated chlorosis strain) [α]
19. *Yersinia pestis* [Ψ]

Fungi

1. *Coccidioides immitis* [χ]
2. *Coccidioides posadasii* [Ψ]
3. *Peronosclerospora philippinensis* [α]
4. *Phakopsora pachyrhizi* [α]
5. *Sclerophthora rayssiae var zeae* [α]
6. *Synchytrium endobioticum* [α]

Exemptions

The following agents or toxins are exempt if the aggregate amount under the control of a principal investigator does not, at any time, exceed:

- 0.5 mg of Botulinum neurotoxins
- 5 mg of *Staphylococcal* enterotoxins
- 100 mg of abrin, *Clostridium perfringens* epsilon toxin, conotoxin, ricin, saxitoxin, shigatoxin, shiga-like ribosome inactivating protein, and tetrodotoxin
- 1,000 mg of diacetoxyscirpenol and T-2 toxin

The following agents or toxins are also exempt:

- Any agent or toxin that is in its naturally occurring environment provided it has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.
- Non-viable select agent organisms or nonfunctional toxins.
- The vaccine strains of Junin virus (Candid #1), Rift Valley fever virus (MP-12), Venezuelan Equine encephalitis virus vaccine strain TC-83.

The medical use of toxins for patient treatment is exempt.

Genetic Elements, Recombinant Nucleic Acids, and Recombinant Organisms

1. Select agent viral nucleic acids (synthetic or naturally derived, contiguous or fragmented, in host chromosomes or in expression vectors) that can encode infectious and/or replication competent forms of any of the select agent viruses.
2. Nucleic acids (synthetic or naturally derived) that encode for the functional form(s) of any of the listed toxins if the nucleic acids: a) are in a vector or host chromosome; b) can be expressed *in vivo* or *in vitro*; or c) are in a vector or host chromosome and can be expressed *in vivo* or *in vitro*.
4. Listed viruses, bacteria, fungi, and toxins that have been genetically modified.

Other Restrictions

1. Experiments utilizing recombinant DNA that involve the deliberate transfer of a drug resistance trait to the listed agents that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.
2. Experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of listed toxins lethal for vertebrates at an LD50 < 100 ng/kg body weight.

Appendix C

Emergency Spill Response

1. Protect Personnel

When a spill incident occurs involving airborne materials in an open area, hold your breath and leave the room immediately. Allow 30 minutes to pass for airborne particles to settle out of the air.

If you are contaminated, wait where you are until help arrives. If you are not contaminated, leave the room or immediate area and wait nearby. Administer first aid when necessary and try to reduce the spread of contamination. Remove contaminated clothing and shoes before leaving the area. Wash face and other contaminated portions of the body with appropriate disinfectant and soap. Use the emergency eyewash and flush for 15 minutes if contaminated material is splashed into the eyes.

Call 911 if immediate medical assistance is required. If immediate medical assistance is not required, ask an uncontaminated co-worker to call the Hazards Control Department for assistance. This, and waiting in the immediate vicinity, will avoid the spread of contamination. If no one is immediately available to assist you, go directly to the nearest telephone and call the area health and safety technician during normal working hours or the off-shift (outside of normal working hours) health and safety technician at ext. 2-7595. If no one responds, call 911. Notify your supervisor and all personnel in the immediate area. Instruct others to stay out of potentially contaminated areas. Post the area(s) that is contaminated.

2. Decontaminate Affected Area

Decontamination of surfaces and equipment is similar to methods used for normal household cleaning. Use of paper towels or wipes with a disinfectant is adequate for most situations. A dilution of 1:10 (1 part household bleach to 9 parts water) will provide 5,000 ppm and is adequate for general decontamination. Wear disposable gloves, laboratory coats or Tyvek coats, and safety glasses as a minimum when decontaminating small objects or areas. Large areas, such as floors or labs, or areas that are difficult to access may require use of additional or special anti-contamination clothing. Consult the area health and safety technician for assistance with the proper selection and use of anti-contamination clothing.

3. Repair or Remove Contaminated Equipment

The ES&H Team shall evaluate contaminated equipment before disassembly for repair or before relocation to a clean area or another facility.